

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Jan Zavada et al.

Group:

Serial No.:

Group Art Unit:

Filed :

Examiner:

For : MN Gene and Protein

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

As part of the accompanying request for filing of a continuation application under 37 CFR 1.53(b)(1), before calculating the filing fee and preliminary to the examination of the above-identified application, please amend the application as indicated below.

IN THE CLAIMS

Please cancel Claims 3-5, 7-12, 18-23 and 26-34.

IN THE SPECIFICATION

Please make the amendments to the Specification as indicated below:

Please replace the paragraph on page 1, lines 2-14 with the following paragraph:

This application is a continuation of allowed U.S. Serial No. 08/485,049 (filed June 7, 1995) which is a continuation-in-part of now pending U.S. Serial No. 08/260,190 (filed June 15, 1994), which, in turn, is a continuation-in-part of U.S. Serial No. 08/177,093 (filed December 30, 1993), which issued as U.S. Patent 6,051,226 on April 18, 2000, which is, in turn, a continuation-in-part of U.S. Serial No. 07/964,589 (filed October 21, 1992), which issued as U.S. Patent No. 5,387,676 on February 7, 1995. This application declares priority under 35 USC § 120 from those U.S. applications, and also under 35 USC § 119 from the now pending Czechoslovakian patent application PV-709-92 (filed March 11, 1992).

Please replace the paragraph on page 8, lines 4-12 with the following paragraph:

This invention also concerns nucleic acids which encode MN proteins or polypeptides that are specifically bound by monoclonal antibodies designated M75 that are produced by the hybridoma VU-M75 deposited at the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Virginia 20110-2209 (USA) under ATCC No. HB 11128, and/or by monoclonal

antibodies designated MN12 produced by the hybridoma MN 12.2.2 deposited at the ATCC under ATCC No. HB 11647.

Please replace the paragraph on page 13, lines 4-15 with the following paragraph:

A hybridoma that produces a representative MN-specific antibody, the monoclonal antibody M75 (Mab M75), was deposited at the ATCC under Number HB 11128 as indicated above. The M75 antibody was used to discover and identify the MN protein and can be used to identify readily MN antigen in Western blots, in radioimmunoassays and immunohistochemically, for example, in tissue samples that are fresh, frozen, or formalin-, alcohol-, acetone- or otherwise fixed and/or paraffin-embedded and deparaffinized. Another representative MN-specific antibody, Mab MN12, is secreted by the hybridoma MN 12.2.2, which was deposited at the ATCC under the designation HB 11647.

Please replace the paragraph on page 16, lines 3-10 with the following paragraph:

The immunoassays of this invention can be embodied in test kits which comprise MN proteins/polypeptides and/or MN-specific antibodies. Such test kits can be in solid phase formats, but are not limited thereto, and can also be in liquid phase format, and can be based on immunohistochemical assays,

ELISAs, particle assays, radiometric or fluorometric assays either unamplified or amplified, using, for example, avidin/biotin technology.

Please replace line 4 on page 18 with the following line:

IPTG - isopropyl-beta-D-thiogalacto-pyranoside

Please delete line 22 on page 19 which reads "SSDS - synthetic splice donor site"

Please replace the paragraph on page 31, lines 11-16 with the following paragraph:

Figure 26 shows a CpG-rich island of a human MN gene. Each vertical line on the scale represents a CpG doublet (upper map) or a GpC doublet (lower map). CpG is 4-5 fold deficient in comparison to GpC, except the island region where the frequency increases about 5 time. CpG and GpC frequencies are roughly equal in the island region.

Please replace the paragraph beginning on page 42, line 19 through page 43, line 2 with the following paragraph:

The details of isolating genomic clones covering the complete genomic region for MN are provided below. Figure 27 provides a schematic of the alignment of MN genomic clones according to the transcription initiation site. Plasmids

containing the A4a clone and the XE1 and XE3 subclones were deposited at the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Virginia 20110-2209 (USA) on June 6, 1995, respectively under ATCC Deposit Nos. 97199, 97200, and 97198.

Please replace the paragraph beginning on page 47, line 21 to page 48, line 7 with the following paragraph:

RNase protection assays (RNP) were performed using Lysate RNase Protection Kit (USB/Amersham) according to protocols of the supplier. Briefly, cells were lysed using Lysis Solution at concentration of approximately 10^7 cells/ml, and 45 μ l of the cell homogenate were used in RNA/RNA hybridization reactions with 32 P-labeled RNA probes prepared as described above. Following overnight hybridizations at 42°C, homogenates were treated for 30 min at 37°C with RNase cocktail mix. Protected RNA duplexes were run on polyacrylamide/urea denaturing sequencing gels. Fixed and dried gels were exposed to X-ray film for 24 - 72 hours.

Please replace the paragraph beginning on page 48, line 9 to page 49, line 2 with the following paragraph:

An RNase protection assay, as described above, was also used to verify the 3' end of the MN cDNA. That was important with respect to our previous finding that the cDNA contains a

poly(A) signal but lacks a poly(A) tail, which could be lost during the proposed degradation of MN mRNA due to the presence of an instability motif in its 3' untranslated region. RNP analysis of MN mRNA with the fragment of the genomic clone XE3 covering the region of interest corroborated our data from MN cDNA sequencing, since the 3' end of the protected fragment corresponded to the last base of MN cDNA (position 10,752 of the genomic sequence). That site also meets the requirement for the presence of a second signal in the genomic sequence that is

needed for transcription termination and polyadenylation [McLauchlan et al., Nucleic Acids Res., 13: 1347 (1985)]. Motif TGTGTTAGT (nt 10,759-10,767) corresponds well to both the consensus sequence and the position of that signal within 22 bp downstream from the polyA signal (nt 10,737-10,742).

Please replace the two paragraphs beginning on page 49, line 4 to page 50, line 13 with the following paragraphs:

The Bd3 genomic clone isolated from human fetal brain cosmid library was found to cover a region of 3.5 kb upstream from the transcription start site of the MN gene. It contains no significant coding region. Two Alu repeats are situated at positions -2587 to -2296 [SEQ. ID NO.: 59] and -1138 to -877 [SEQ. ID NO.: 60] (with respect to the transcription start determined by RNP). The sequence proximal to the 5' end is

strongly homologous (91.4% identity) to the U3 region of long terminal repeats of human endogenous retroviruses HERV-K [Ono, M., "Molecular cloning and long terminal repeat sequences of human endogenous retrovirus genes related to types A and B retrovirus genes," J. Virol., **58**: 937-944 (1986)]. The LTR-like fragment is 222 bp long with an A-rich tail at its 3' end. Most probably, it represents part of SINE (short interspersed repeated sequence) type nonviral retroposon derived from HERV-K [Ono et al., "A novel human nonviral retroposon derived from an endogenous retrovirus," Nucleic Acids Res., **15**: 8725-8373 (1987)]. There are no sequences corresponding to regulatory elements in this fragment, since the 3' part of U3, and the entire R and U5 regions of LTR are absent from the Bd3 genomic clone, and the glucocorticoid responsive element as well as the enhancer core sequences are beyond its 5' border.

However, two keratinocyte-dependent enhancers were identified in the sequence downstream from the LTR-like fragment at positions -3010 and -2814. Those elements are involved in transcriptional regulation of the E6-E7 oncogenes of human papillomaviruses and are thought to account for their tissue specificity [Cripe et al., "Transcriptional regulation of the human papillomavirus-16 E6-E7 promoter by a keratinocyte-dependent enhancer, and by viral E2 trans-activator and repressor

gene products: implications for cervical carcinogenesis," EMBO J., 6: 3745-3753 (1987)].

Please replace the paragraph on page 65, lines 5-11 with the following paragraph:

Where the host used is an eukaryote, transfection methods such as the use of a calcium phosphate-precipitate, electroporation, conventional mechanical procedures such as microinjection, insertion of a plasmid encapsulated in red blood cell ghosts or in liposomes, treatment of cells with agents such as lysophosphatidyl-choline or use of virus vectors, or the like may be used.

Please replace the paragraph beginning on page 72, line 21 to page 73, line 2 with the following paragraph:

The MN 20-19 protein was purified from the conditioned media by immunoaffinity chromatography. 6.5 mg of Mab M75 was coupled to 1 g of Tresyl activated Toyopearl™ [Tosoh, Japan (#14471)] (solid support in bead form). Approximately 150 ml of the conditioned media was run through the M75-Toyopearl™ (solid support in bead form) column. The column was washed with PBS, and the MN 20-19 protein was eluted with 1.5 M MgCl₂. The eluted protein was then dialyzed against PBS.

Please replace the paragraph on page 101, lines 15-21 with the following paragraph:

MAb M75. Monoclonal antibody M75 (MAb M75) is produced by mouse lymphocytic hybridoma VU-M75, which was initially deposited in the Collection of Hybridomas at the Institute of Virology, Slovak Academy of Sciences (Bratislava, Slovak Republic) and was deposited under ATCC Designation HB 11128 on September 17, 1992 at the American Type Culture Collection (ATCC) in Manassas, Virginia (USA).

Please replace the paragraph on page 104, lines 14-25 with the following paragraph:

Mab MN12. Monoclonal antibody MN12 (Mab MN12) is produced by the mouse lymphocytic hybridoma MN 12.2.2 which was deposited under ATCC Designation HB 11647 on June 9, 1994 at the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Virginia 20110-2209 (USA). Antibodies corresponding to Mab MN12 can also be made, analogously to the method outlined above for Mab MN9, by screening a series of antibodies prepared against an MN protein/polypeptide, against the peptide representing the epitope for Mab MN12. That peptide is AA 55 - AA 60 of Figure 1 [SEQ. ID. NO.: 11]. The Novatope system could also be used to find antibodies specific for said epitope.

Please replace the paragraph beginning on page 109, line 23 to page 110, line 10 with the following paragraph:

MN proteins and/or polypeptides may be synthesized or prepared recombinantly or otherwise biologically, to comprise one or more amino acid sequences corresponding to one or more epitopes of the MN proteins either in monomeric or multimeric form. Those proteins and/or polypeptides may then be incorporated into vaccines capable of inducing protective immunity. Techniques for enhancing the antigenicity of such polypeptides include incorporation into a multimeric structure, binding to a highly immunogenic protein carrier, for example, keyhole limpet hemocyanin (KLH), or diphtheria toxoid, and administration in combination with adjuvants or any other enhancers of immune response.

Please replace the paragraph beginning on page 113, line 20 to page 114, line 6 with the following paragraph:

Human sera from cancer patients, from patients suffering with various non-tumor complaints and from healthy women were obtained from the Clinics of Obstetrics and Gynaecology at the Postgraduate Medical School, Bratislava, Slovak Republic. Human serum KH was from a fifty year old mammary carcinoma patient, fourteen months after resection. That serum was one of two sera out of 401 serum samples that contained

neutralizing antibodies to the VSV(MaTU) pseudotype as described in Zavada et al. (1972), supra. Serum L8 was from a patient with Paget's disease. Serum M7 was from a healthy donor.

Please replace the paragraph on page 122, lines 15-22 with the following paragraph:

A radioimmunoassay was performed directly in confluent petri dish (5 cm) culture of cells, fixed with methanol essentially as described in Example 3, supra. The monolayers were fixed with methanol and treated with ^{125}I -labeled MAbs M67 (specific for exogenous MX antigen) or M75 (specific for endogenous MN antigen) at 6×10^4 cpm/dish. The bound radioactivity was measured; the results are shown in Figure 6.

Please replace the paragraph on page 135, lines 3-17 with the following paragraph:

It was found that cultivation of HeLa cells with the ODNs resulted in considerable inhibition of p54/58N synthesis. The 19-mer ODN2 (Figure 3B) in 4 μM final concentration was very effective; as determined by RIA, it caused 40% inhibition, whereas the 29-mer ODN1 (4 μM) (Figure 3A) and a combination of the two ODNs (Figure 3C), each in 2 μM final concentration, were less effective in RIA showing a 25-35% decrease of the MN-related proteins. At the same time, the amount of different HeLa cell

protein determined by RIA using specific MAb H460 was in all cell variants approximately the same. Most importantly was that on immunoblot it could be seen that specific inhibition by the ODNs affected both of the p54/58N proteins. Thus, we concluded that the MN gene we cloned coded for both p54/58N proteins in HeLa cells.

Please replace the three paragraphs beginning on Page 157, line 24 to page 158, line 22 with the following paragraphs:

ATCC Deposits. The material listed below was deposited with the American Type Culture Collection (ATCC) at Manassas, Virginia 20110-2209 (USA). The deposits were made under the provisions of the Budapest Treaty on the International Recognition of Deposited Microorganisms for the Purposes of Patent Procedure and Regulations thereunder (Budapest Treaty). Maintenance of a viable culture is assured for thirty years from the date of deposit. The hybridomas and plasmids will be made available by the ATCC under the terms of the Budapest Treaty, and subject to an agreement between the Applicants and the ATCC which assures unrestricted availability of the deposited hybridomas and plasmids to the public upon the granting of patent from the instant application. Availability of the deposited strain is not to be construed as a license to practice the invention in

contravention of the rights granted under the authority of any Government in accordance with its patent laws.

<u>Hybridoma</u>	<u>Deposit Date</u>	<u>ATCC #</u>
VU-M75	September 17, 1992	HB 11128
MN 12.2.2	June 9, 1994	HB 11647

<u>Plasmid</u>	<u>Deposit Date</u>	<u>ATCC #</u>
A4a	June 6, 1995	97199
XE1	June 6, 1995	97200
XE3	June 6, 1995	97198

IN THE DRAWINGS

Please replace Sheet 5 containing Figures 4 and 5 with the new Sheet 5. The replacement sheet is necessary to correct Figure 5.

REMARKS

To assist in the examination of this application and as required by 37 CFR 1.121, enclosed herewith as Appendix 1 is a marked up version of the changes made to the specification to indicate how the previous version of the specification has been modified to produce the clean replacement paragraphs. The

modifications are indicated by underlining and in bold type for additions, and by strikeouts for deletions.

Specification

Amendments to the Specification were made at page 1 to update the status of the priority applications. The Specification is amended on pages 8, 42, 101, 104 and 157 to update the address of the American Type Culture Collection (ATCC) which has been relocated. American Type Culture Collection (ATCC) deposit information concerning the plasmids A4a, XE1 and XE3 is added to the Specification at pages 42-43 and 158.

Typographical/proofreading errors have been corrected at pages 13, 16, 18, 19, 31, 47-50, 65, 109-110, 113-114, 122 and 135. "SSDS - synthetic splice donor site" inadvertently appeared twice in the abbreviation list, the first time (at page 19, line 22) out of alphabetical order; so the initial rendition of that phrase was deleted.

A generic phrase for the trademarked product "Toyopearl™" is added at pages 72-73

Replacement of SEQ ID NOS: 56 and 57 with SEQ ID NOS: 59 and 60

The Specification is amended at page 49, lines 8 and 9 to correct proofreading/typographical errors to replace SEQ ID

NOS: 56 and 57 with SEQ ID NOS: 59 and 60. SEQ ID NOS: 56 and 57 have no sequences assigned to them as they are duplicated by SEQ ID NOS: 59 and 60. Proofreading oversights caused the use of two sequence ID numbers for the same sequence.

As indicated at page 49, lines 7-9, SEQ ID NO: 56 had been given for the Alu repeat at positions -2587 to -2296, and SEQ ID NO: 57 had been given to the sequence Alu repeat at -1138 to -877. Then at page 84, lines 5 and 6, it is indicated that SEQ ID NO: 59 was at nucleotides 921 to 1212 and SEQ ID NO: 60 was at nucleotides 2370 to 2631. SEQ ID NO: 56 had been the same as SEQ ID NO: 59, and SEQ ID NO: 57 had been the same as SEQ ID NO: 60. The oversights occurred in that there are two different ways of designating the same sequence, one as per Figure 15A-D, and one as per nucleotide 3507 being the transcription initiation site.

Replacement of the Word "Increase" with the Word "Decrease" at Page 135, Line 10

For the correction at page 135, line 10, it can be seen in Figure 3 and in the description of Figure 3 at page 24, line 21 to page 25, line 2, that the antisense oligonucleotides inhibited MN protein (p54/58) expression in lanes A, B and C in comparison to the control lane D. Although Figure 3 shows that the most inhibition (40%) occurred in lane B, that lanes A and B

show a 25-35% inhibition of p54/58 expression. Applicants therefore respectfully submit that it is clear from the context of Example 10 (page 134, line 6 to page 135, line 23) in combination with Figure 3 and its description, that the use of the word "increase" at page 135, line 10 was in error and should have read "decrease".

Replacement of Figure 5 of the Drawings

The replacement of Figure 5 of the drawings is necessary to replace the erroneous Figure 5, which was inadvertently filed with the priority application U.S. Serial No. 08/485,049 due to a clerical error. The Applicants did not recognize that error prior to the application being filed because Figures 2-14 were supposed to be the same figures as in the two preceding priority applications -- now U.S. Patent No. 6,051,226 and now pending U.S. Serial No. 08/260,190.

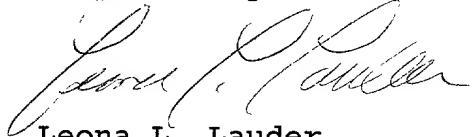
Applicants respectfully point out that U.S. Patent No. 6,051,226 and U.S. Serial No. 08/260,190, from which the priority application (U.S. Serial No. 08/485,049) claims priority, contain the correct Figure 5, a copy of which is supplied herewith. Figure 5 is described in the Specification on page 25, lines 9-14 and in Example 12, specifically at page 137, lines 4-20. The descriptions of Figure 5 in the instant application are the same

as in the claimed priority applications, but the version of Figure 5 in the instant application does not match Figure 5 of the priority applications -- U.S. Patent 6,051,226 and pending U.S. Serial No. 08/260,190.

CONCLUSION

Applicants respectfully submit that the above amendments do not add any new matter and request that they be entered for the instant application. If the undersigned Attorney for the Applicants can be of any assistance in regard to this Preliminary Amendment, she can be reached at (415) 981-2034.

Respectfully submitted,



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Dated: January 30, 2001